[0066] The breeding values of the bulls are centrally estimated by the United Information Systems Animal Production (Vereinigte Informationssysteme Tierhaltung—VIT) in Verden. A total amount of more than 150,000 daughters and their performance data are integrated in the estimation of the breeding values. From all bulls, deregressed breeding values, concerning the milk yield, the protein and fat yield, the protein content (in %), are utilized in the variance component estimation. The deregression of the breeding values is carried out as described by Thomsen et al. (2001, J Anim Breed Genet. 118, 357-370).

[0067] The variance component estimation is carried out using the program package SAS. First, as unique fixed effect, the marker CSN1S1 is considered in the model, because other influence factors (e.g. operational effects, milking frequency) are already corrected in the frame of the estimation of the breeding value and the deregression (influence of the sires). The analysis reveals significant effects of the marker CSN1S1 on all studied traits (deregressed breeding values for protein percentage (DRG\_PP), milk yield (DRG\_MY1), fat yield (DRG\_FY1), protein yield (DRG\_PY1), fat percentage (DRG\_FP)). Table 3 shows the effect of CSN1S1 on deregressed breeding values for milk production traits, indicating also the probability of error (p) for the effects on the individual traits.

TABLE 3

Probability of error (p)	
<0.0001	
0.0011	
0.0016	
0.0056	
0.0052	

[0068] The highest significance is calculated for the effect on DRG\_PP. As the examined marker CSN1S1 is located directly within the regulatory region of a milk protein gene, this could be an indication of a direct effect. The marker CSN1S1 fulfils the requirements to a functional candidate gene.

[0069] The highest breeding value for milk (DRG\_MY1) is achieved on average by bulls with the genotype 12, whereas the highest breeding values for protein percentage (DRG\_PP) are found within the group with genotype 24. Table 4 shows a compilation of the least square means (LS\_means) for the groups with the genotypes 12, 22, 23 and 24. The table displays the LS\_means as well as standard errors for the deregressed breeding values for milk yield (DRG\_MY1) and protein percentage (DRG\_PP) in groups with different CSN1S1 genotypes.

TABLE 4

CSN1S1		LSMEAN ± se				
type	n	DRG_MY1	DRG_PP			
12	79	198.232 ± 15.700	-0.00022534 ± 0.00006470			
22	398	155.341 ± 6.995	-0.00037495 ± 0.00002921			
23	131	138.806 ± 12.192	-0.00038405 ± 0.00005271			
24	76	112.364 ± 16.007	$0.00008175 \pm 0.00006650$			
Alle	684	152.353	-0.000307			

[0070] In order to obtain a more exact clarification, the variance analysis is repeated within individual families and groups of families with identical genotypes. Hereby is revealed, that the effect on the milk yield can not be confirmed in all families. In family 9, in which the sires exclusively passed down the allele 2, the only remaining effect is encountered close to the 5% threshold of significance for DRG\_PP (p=0.0610). Furthermore, a comparison of the LS\_means for the traits DRG\_MY1, DRG\_PP, DRG\_FP is carried out for all groups of genotypes and within each individual family, and it is proved whether the difference of the LS-means between the genotypes 12, 23 and 24 and the most frequent genotype 22 is significant. The results are graphically illustrated in FIG. 5.

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<301> AUTHORS: Koczan Dirk, Hobom Gerd, Seyfert Hans-Martin
<302> TITLE: Genomic organization of the bovine alpha S1-casein gene
<303> JOURNAL: Nucleic acids research
<304> VOLUME: 19
<305> ISSUE: 20
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- 1. Genetic marker at the 5'-flanking region of the  $\alpha S1$  casein gene (CSN1S1) characterized by the fact that it contains the nucleotide sequence 1-1061, preferably the nucleotide sequence 1-655 at the 5'-flanking region of the  $\alpha S1$  casein gene.
- Genetic marker according to patent claim 1 characterized by its amplification by means of PCR reaction either through

```
Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 2
CSN1S1pro1r (5' GAA GAA GCA GCA AGC TGG 3')

or through

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')
```

- 3. Genetic marker according to patent claim 1 characterized by its variability within milk breeds.
- 4. Genetic marker according to patent claim 1 characterized by its utilization in order to determine the allelic state at the 5'-flanking region of the  $\alpha$ S1 casein gene.
- 5. Procedure to determine the allelic state of the 5'-flanking region of the  $\alpha s1$  casein gene, characterized by the following steps:
  - a) provision of the source material of the organism to be examined
  - b) isolation of the genetic material
  - c) targeted isolation or enrichment of the marker fragment at the 5' region of the as1 casein gene or of a sequence, which contains portions of the marker sequence, preferably the fragment 1 to 655 of the marker sequence out of the as1 casein gene
  - d) Proof of the allelic state in the isolated or enriched sequence fragment of the marker fragment of the as1 casein gene.
- 6. Procedure according to patent claim 5 characterized by the utilization of source material coming from an animal, particularly a mammal, in particular a bovine, a sheep or a goat, including breed animals and embryos of these species.

- 7. Procedure according to patent claim 5 characterized by the utilization of blood, leukocytes, tissue including biopsy material, milk, sperm, hair, individual cells including cell material from embryos, a bacteria culture or isolated chromosomes as source material.
- 8. Procedure according to patent claim 5 characterized by the utilization of source material coming from a genetically modified organism (GMO) which contains the marker fragment of the as1 casein gene.
- 9. Procedure according to patent claim 5 characterized by the utilization of genetic material containing genomic DNA or RNA from animals, plasmid DNA from bacteria, from artificial chromosomes such as BACs and YACs.
- 10. Procedure according to patent claim 5 characterized by achieving the enrichment of the marker segment of the asl casein gene by means of polymerase chain-reaction.
- 11. Procedure according to patent claim 5 characterized by the enrichment of the marker segment of the \alphas1 casein gene by means of polymerase chain-reaction with the oligonucleotides

```
Primer 1
CSN1S1prolf (5' GAA TGA ATG AAC TAG TTA CC 3')
Primer 2
CSN1S1prolr (5' GAA GAA GCA GCA AGC TGG 3')
Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')
```

as primers, whereby the following combinations are selected: primer 1 with primer 2 and primer 2 with primer 3.

- 12. Procedure according to patent claim 5 characterized by the determination the allelic state by means of SSCP, RFLP, OLA, TGGE, ASPCR, PCR-ELISA, microarray method or through nucleic acid sequencing.
- 13. Procedure according to patent claim 5 characterized by detection of one or more of the allelic states of the marker sequence of the  $\alpha s1$  casein gene.
- 14. Utilization of the procedure according to claim 5 in order to examine the animals' milk production traits, independently of age and lactation.
- 15. Utilization of the procedure according to claim 5 in order to select organisms which carry a certain allelic state or a certain genotype of the marker sequence of the asl casein gene or a portion thereof.
- 16. Utilization of the procedure according to claim 5 in breeding programs, particularly for a marker-supported selection.